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# Efficacy of Soil Amendments with Organic Manures, *Lantana camara* and Microalgae Against *Alternaria alternata* of Stevia (*Stevia rebaudiana*)

***V. Praveen Kumar* and Sobita Simon***
*Department of Plant Pathology,*
*Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, Prayagraj, (UP) India.*

*(Corresponding author: V. Praveen Kumar*)*
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**ABSTRACT: *Stevia rebaudiana* (Bertoni) is a herbaceous perennial plant of the Asteraceae family, native to Paraguay (South America). It is also known as the "sweetest plant of the world". Stevia is a semi-humid subtropical plant, is prone to Alternaria leaf spot, a major foliar disease caused by *Alternaria alternata*. Managing this fungal disease is crucial for optimal stevia cultivation. In a field experiment at SHUATS, Prayagraj, various organic manures, including Farmyard Manure (FYM), spent mushroom compost (SMC), neem cake (NC), *Lantana camara*, and microalgae, were evaluated for their effectiveness against alternaria leaf spot, growth, and yield of stevia. Among the ten treatments, $T_9$ – FYM + SMC + NC + *Lantana camara* + microalgae significantly reduced disease intensity at 45 and 90 days after treatment (10.65% and 23.32%). This treatment also excelled in plant height at 30, 60, and 90 days after treatment (59.76 cm), with the maximum number of suckers and fresh and dry leaf weights recorded at 45 and 90 days. The results highlight the potential of combining organic manures to reduce disease intensity while enhancing stevia growth and yield. The experiment took place during the Rabi season of 2021-2022.**

**Keywords:** *Alternaria alternata*, Farmyard manure, *Lantana camara*, Neem Cake, Microalgae, Spent Mushroom Compost.

## INTRODUCTION

*Stevia rebaudiana* (Bertoni) is a herbaceous perennial plant of the Asteraceae family, native to Paraguay (South America). Stevia, often referred to as the "sweetest plant in the world," is a remarkable herb known for its intense natural sweetness. In cultivation, these plants can attain heights exceeding 1 meter, although they typically reach a more manageable height of 60–70 cm. Stevia thrives in a semi-humid subtropical climate, making it adaptable for cultivation in kitchen gardens. Ideal soil conditions for stevia include well-drained red soil and sandy loam soil, maintaining a pH range of 6.5 to 7.5. The plant exhibits resilience to a temperature range of 6 to 43 degrees Celsius, with an average temperature of 23 degrees Celsius, and it flourishes with an annual rainfall between 1500 to 1800mm (Ashok *et al.*, 2011). These specific environmental preferences highlight the versatility and adaptability of stevia, making it a suitable candidate for cultivation in various regions with the right conditions.

The main constituents present were glycosides such as stevioside, steviol and rebaudioside A and B. The two main alkaloids being stevioside (ST) and rebaudioside A (R-A) are the sweetest compounds and tasting about 300 and 450 times sweeter than sucrose, respectively (Surjit *et al.*, 2012). Dry leaves of stevia are sweeter approximately 10 to 15 times than sucrose while glycemic index is zero. The fresh Stevia leaves contain a large amount of water between 80 and 85%.

The Stevia plant and its extracts have emerged as a versatile solution in combating various health issues, including obesity, cavities, hypertension, fatigue, depression, and yeast infections. According to Dushyant *et al.* (2014), Stevia exhibits an array of beneficial properties such as hypoglycemic, hypotensive, vasodilating, taste-improving, sweetening, anti-fungal, anti-viral, anti-inflammatory, and anti-bacterial effects. Additionally, it enhances the body's urination function. The Stevia glycosides (SvGls) content follows a slightly different order of decline: leaves > shoots > roots > flowers. Currently, China is the primary producer of Stevia, with Japan leading the market for its diverse applications. Stevia's multifaceted health benefits and its natural sweetness make it an increasingly popular choice as a sugar substitute and a valuable component in various health-promoting products.

The prevalence of fungal diseases, particularly Alternaria leaf spot caused by *Alternaria alternata* in stevia, has been a significant concern. Alternaria leaf spot stands out as a major foliar disease impacting the leaves, the primary economic component of the stevia plant. A five-year survey has consistently identified Alternaria leaf disease as a common issue in medicinal plants. The symptoms manifest initially as small circular spots with a light brown hue, evolving into irregular shapes with dark brown to grey tones. Some spots maintain a circular appearance with concentric rings or zones. Severely affected leaves witness the coalescence of multiple spots, forming extensive necrotic areas. The diameter of these leaf spots varies between 2 to 18 mm. The conidia responsible for the disease display mid to dark brown coloration, are short-beaked, arranged in lengthy chains, and feature an oval and bean-shaped structure with 3-5 transverse septa (Maiti *et al.*, 2007). This comprehensive understanding of the disease's symptoms and characteristics is crucial for implementing effective management strategies in stevia cultivation.

## MATERIALS AND METHODS

The experimentation involved one-year-old stevia plants, pre-existing in the Central Research Field of the department of plant pathology at SHUATS, Allahabad. These plants were previously afflicted with Alternaria leaf spot. The experimental design employed a randomized block structure, comprising five replications to ensure robustness and reliability of the results. For analysis, leaf samples were meticulously collected from the infested field and subjected to identification processes under compound microscope.

The designated field underwent meticulous weeding, and the dried offshoots of each individual plant were carefully separated from the parent plant. Subsequently, the entire area was divided into five replications, each with a plot size of 1×1m, utilizing a randomized block design. This design incorporated ten distinct treatments for the experimental purposes. These treatments included the application of 4kg of Farm Yard Manure (FYM) per plot, 50gm of Spent Mushroom Compost per plot, 50gm of Neem Cake per plot, and 50gm of *Lantana camara* per plot, all utilized as basal soil applications. The application of these treatments involved incorporating them into the soil at the rhizosphere area of the plants, followed by a covering of a thin layer of soil. To facilitate the decomposition process, the field underwent irrigation at regular intervals for a duration of fifteen days. After a lapse of thirty days, a solution containing 500 grams of microalgae blended with 10 liters of water was prepared, followed by the careful application of 10 grams per plot of microalgae to each individual plot. Subsequently, at day 60 post-treatment, a second dose of microalgae mirroring the initial 10 grams per plot application was administered. The growth parameters of stevia plants were systematically documented at intervals of 30, 60, and 90 days post-treatment. Furthermore, disease intensity and the yield of dry leaves were methodically recorded at 45 and 90 days after the treatment of the crop. All collected records have undergone rigorous statistical analysis for a comprehensive evaluation of the outcomes.

Disease infection became apparent on leaves at the 35-day mark following the treatment in the control plot. The assessment of disease intensity was conducted at both 45 and 90 days after treatment. The percentage of disease intensity was quantified using a rating scale ranging from 0 to 5, as stipulated by Shahzad and Bhat (2005).

**Table 1: 0 to 5 rating scale for measuring disease intensity.**

| Category | Numerical value | Leaf area infected % |
|---|---|---|
| I | 0 | Disease free |
| II | 1 | 0.1 - 10 |
| III | 2 | 10.1 – 25 |
| IV | 3 | 25.1 – 50 |
| V | 4 | 50.1 -75 |
| VI | 5 | >75 |

$$\text{PDI} = \frac{\sum (n \times v)}{N \times G} = 100$$

where, Σ=Summation;
n=Number of leaves in each category;
v=Numerical value of each category;
N=Total Number of leaves examined;
G=Maximum numerical value

Infected leaves, exhibiting typical leaf spot symptoms, were gathered from the central research field and subjected to microscopic observation for fungal spores. The isolation process followed the standard tissue isolation technique, involving the collection of infected leaves cut into small pieces. These pieces were surface sterilized using 0.1% mercuric chloride for one minute, followed by a sterilization step using distilled water. The sterilized fragments were aseptically transferred into petri plates containing slants with potato dextrose agar media. The setup was then incubated at room temperature (25 ± 10ºC) for a period of 3-6 days, with regular observations made for fungal growth. Identification of the pathogen was based on a thorough analysis of morphological and cultural characteristics.

**Fig. 1.** Sympotoms of Alternaria leaf spot.

**Fig. 2.** Slants showing the growth of *Alternaria alternata*.

## RESULT AND DISCUSSION

The findings presented in Table 1 indicate that all treatments exhibited statistical significance, leading to a reduction in Alternaria leaf spot in stevia compared to the control. Notably, among the treatments, $T_9$ - FYM + Neem cake + SMC + *Lantana camara* + Microalgae demonstrated a substantial decrease in disease intensity (10.65%), signifying its effectiveness. Certain treatments, such as ($T_7$, $T_8$), ($T_4$, $T_5$), ($T_6$, $T_2$), and ($T_2$, $T_3$), were found to be non-significant when compared to each other. Additionally, the treatment $T_9$, which incorporates FYM + Neem cake + SMC + *Lantana camara* + Microalgae (23.32%), significantly lowered the disease intensity of okra when contrasted with other treatments. Conversely, treatments ($T_8$, $T_4$), ($T_5$, $T_6$), and ($T_2$, $T_3$) did not exhibit significant differences among themselves.

**Table 2: Evaluation of the soil amendments with bio-resources on disease intensity (%) of *Alternaria alternata* of stevia at 45 and 90 DAT.**

| Sr. No. | Treatment details | 45 DAT | 90 DAT |
|---|---|---|---|
| $T_0$ | Control (FYM) + Microalgae | 22.30 | 46.03 |
| $T_1$ | SMC + Microalgae | 19.97 | 42.31 |
| $T_2$ | *Lantana camara* + Microalgae | 17.67 | 38.51 |
| $T_3$ | Neem cake + Microalgae | 18.48 | 39.94 |
| $T_4$ | FYM + *Lantana camara* + Microalgae | 15.13 | 30.76 |
| $T_5$ | SMC + Neem cake + Microalgae | 15.94 | 33.44 |
| $T_6$ | Neem cake + *Lantana camara* + Microalgae | 17.32 | 35.28 |
| $T_7$ | FYM + SMC + *Lanatana camara* + Microalgae | 12.37 | 26.06 |
| $T_8$ | FYM + Neem cake + *Lantana camara* + Microalgae | 13.10 | 29.45 |
| $T_9$ | FYM + Neem cake + SMC + *Lantana camara* + Microalgae. | 10.65 | 23.32 |
| F – test | | S | S |
| SE (d)± | | 0.71 | 1.55 |
| CD (0.05) | | 1.14 | 2.05 |

**Table 3: Evaluation of the soil amendments with bio-resources on growth parameters and yield of stevia.**

| Treatments | Plant height (cm) | No. of suckers | Fresh leaves yield (gm) | Dry leaves yield (gm) |
|---|---|---|---|---|
| **$T_0$** | 44.09 | 35.60 | 25.38 | 17.38 |
| **$T_1$** | 46.38 | 41.60 | 28.88 | 19.08 |
| **$T_2$** | 48.33 | 44.80 | 30.28 | 20.28 |
| **$T_3$** | 47.34 | 42.40 | 29.97 | 19.97 |
| **$T_4$** | 54.17 | 51.80 | 32.35 | 22.35 |
| **$T_5$** | 51.74 | 50.20 | 31.68 | 21.68 |
| **$T_6$** | 50.18 | 48.60 | 31.06 | 20.66 |
| **$T_7$** | 58.12 | 55.80 | 34.07 | 23.45 |
| **$T_8$** | 56.17 | 55.20 | 33.57 | 23.17 |
| **$T_9$** | 59.75 | 58.80 | 36.73 | 25.33 |
| **F- test** | S | S | S | S |
| **S. E (d) ±** | 0.81 | 1.43 | 1.10 | 0.66 |
| **C.D. (5%)** | 1.63 | 2.89 | 2.23 | 1.34 |

**Evaluation of bio resources on growth parameters plant height (cm) and number of suckers of stevia at 90 DAT.** The results presented in Table 3 demonstrate the statistical significance of all treatments in positively impacting the plant growth parameters of stevia. Notably, among the treatments, $T_9$ – FYM + Neem cake + SMC + *Lantana camara* + Microalgae stood out by significantly increasing the plant height of stevia (59.75 cm). Conversely, treatments ($T_1$, $T_3$), ($T_2$, $T_3$), and ($T_5$, $T_6$) were found to be non-significant when compared to each other in terms of plant height. Furthermore, $T_9$ – FYM + Neem cake + SMC + *Lantana camara* + Microalgae (58.80) significantly elevated the number of suckers in stevia. Treatments ($T_1$, $T_3$), ($T_3$, $T_2$), ($T_6$, $T_5$), ($T_5$, $T_4$), and ($T_8$, $T_7$) did not exhibit significant differences among themselves in terms of the number of suckers. These findings underscore the positive impact of $T_9$ on the growth parameters of stevia compared to other treatments.

**Evaluation of effect of treatments on fresh and dry leaves yield(gm) of stevia at 90 DAT**. The data presented in Table 3 indicates the statistical significance of all treatments in enhancing the fresh leaves yield of stevia. Notably, $T_9$ – FYM + Neem cake + SMC + *Lantana camara* + Microalgae exhibited a significant increase in fresh leaves yield (36.73 gm) for stevia. Conversely, various treatment groups, including ($T_1$, $T_3$, $T_2$), ($T_2$, $T_6$, $T_5$), ($T_6$, $T_5$, $T_4$), ($T_5$, $T_4$, $T_8$), and ($T_8$, $T_7$), did not show significant differences in fresh leaves yield when compared to each other. Moreover, $T_9$ – FYM + Neem cake + SMC + *Lantana camara* + Microalgae (25.33 gm) significantly elevated the dry leaves yield of stevia, with treatments ($T_1$, $T_3$, $T_2$), ($T_2$, $T_6$, $T_5$), ($T_5$, $T_4$), and ($T_4$, $T_8$, $T_7$) demonstrating non-significant variations among themselves. These results underscore the substantial positive impact of $T_9$ on the fresh and dry leaves yield of stevia compared to other treatments.

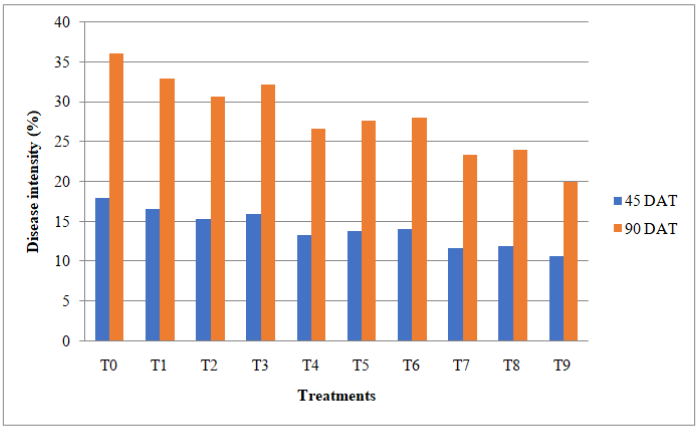


**Fig. 3.** Evaluation of bio resources on disease intensity of alternaria leaf spot of stevia.

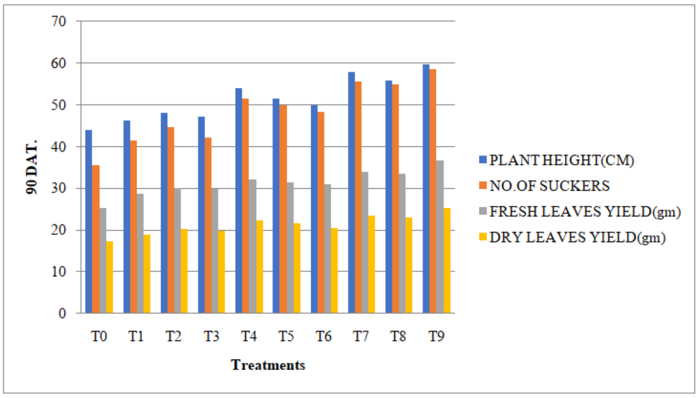


**Fig. 4.** Evaluation of bio resources on plant height, number of suckers, fresh leaves yield and dry leaves yield of stevia at 90 DAT.

## CONCLUSIONS

The current field experimental investigation reveals that the most substantial reduction in disease occurred with the combination treatment $T_9$ – incorporating Farm Yard Manure (FYM), neem cake, Soil Moisture Conservation (SMC), *Lantana camara*, and Microalgae, showing a noteworthy decrease of 23.32%, as opposed to the control group (46.03%). Consequently, it can be deduced that the application of bio resources effectively mitigates foliar Alternaria leaf spot, diminishes disease intensity, and enhances the overall growth of stevia.

## FUTURE SCOPE

The integration of organic manures with *Lantana camara* and microalgae in stevia cultivation holds significant promise for sustainable disease management. Investigating the long-term impacts on soil health and microbial communities will provide valuable insights, highlighting the potential of this approach as an environmentally friendly and economically viable strategy against alternaria leaf spot in stevia cultivation. This comprehensive exploration aims not only to enhance disease resistance but also to establish a holistic and sustainable framework for stevia cultivation practices, aligning with principles of ecological balance and economic viability.

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